

Nawrocki 5.21.2010

ISOLATING DNA FROM TRIZOL LAYERS

***After addition of BCP or Phenol and subsequent removal of aqueous phase for RNA extraction, an interphase (milky white, usually) and bottom organic phase (pink) will be left. This is stored at 4 degrees for later DNA isolation. This protocol picks up from there...**

- 1) Add 300 ul 100% Ethanol (200 proof EtOH) per 1 ml TRIZOL used to interphase/organic phase.
- 2) Vortex gently.
- 3) Incubate samples at 15-30°C for 2-3 minutes.
- 4) Centrifuge at 12,000 RPM for 5 minutes at room temp.

DNA Wash:

- 1) Remove phenol/ethanol sup (contains protein) to waste. (Pipet into 50ml conical & dispose in Trizol Waste container)
 - 2) Wash DNA pellet two times in 0.1M sodium citrate solution. Add 1 ml Na citrate per 1 ml Trizol used.
 - 3) Gently vortex. Incubate samples in wash solution at 15-30°C for 30 minutes (periodic mixing).
 - 4) Spin at 12,000 RPM for 5 minutes at room temp.
 - 5) After washes resuspend DNA in 1 ml 75% Ethanol per 1 ml TRIZOL used. USE: 200 proof EtOH and PCRgrade water. (eg. For 50ml, 37.5ml of ethanol and 12.5ml of water)
 - 6) Incubate at 15-30°C for 10-20 minutes (periodic mixing).
 - 7) Centrifuge at 12,000 RPM for 5 minutes at room temp. Remove sup using p200 pipettor.
 - 8) Briefly dry pellet for 2-5 minutes under vacuum. (Can leave on bench)
- *Add 100µl of water and freeze at -80 or continue to next step.

Redissolving the DNA:

- 1) Dissolve pellet in 100ul (or 200-500ul for thymus) of sterile water with a pipette. Can place sample at 55°C for 10 minutes to increase solubility.
*Solution may still contain insoluble fragments (membrane, protein, etc).
- 2) Centrifuge samples at 12,000 RPM for 10 minutes in cold room to remove insoluble material.
- 3) Transfer sup containing DNA to a new, autoclaved, labeled, flip-top eppendorf.
- 4) Spec. AMOUNT: 1ul of sample + 50ul of dH₂O (198ul H₂O+2ul sample)
- 5) Store at -80.